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TESTS FOR INDEPENDENCE OF WALTZER AND EP SONOGENIC CONVULSIVE FROM CERTAIN OTHER GENES IN THE DEER MOUSE (PEROMYSCUS MANICULATUS)

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INTRODUCTION

PREVIOUS studies of linkage relations in the species Peromyscus maniculatus (Sumner, 1922; Feldman, 1937; Clark, 1936, 1938; Barto, 1942; Huestis and Piestrak, 1942; Huestis and Lindstedt, 1946; McIntosh, 1956) permit the tentative assignment of eight genes to four linkage groups, although the independence of one of these groups from the other three needs further testing (McIntosh, 1956). Two other genes, wide-band and waltzer, are linked with 11 per cent crossing over (McIntosh, 1956). Independence of one or the other of these two genes from one gene in each of the four numbered linkage groups has already been demonstrated (Clark, 1938; Barto, 1942; McIntosh, 1956). The present report gives the results of five additional tests with waltzer which complete the evidence necessary to establish this gene and wide-band as markers of a fifth linkage group. Three tests of independence involving the EP syndrome are also reported.

Acknowledgments

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METHODS

Seven of the tests for independence reported here were made on testcross data; one was made on an F_2 generation. Five of the tests are based on both repulsion and coupling crosses; three, on crosses in the repulsion phase only. In each experiment the age of the segregating parents was at least three months when the first litter was born, and more than half of the offspring came from matings in which the doubly heterozygous parent was more than twelve months old. Unless otherwise noted the progeny were classified when about one month old.

The data were analyzed according to methods described by Mather (1951). Heterogeneity for the segregation of the single factors and for the proportion of recombinant phenotypes between phases and between segregating sexes within phases of the testcrosses was tested by means of chi square, using the Brandt-Snedecor formula for a hierarchical classification (Mather, 1951, sec. 7). Heterogeneity among sibships was also tested as far as this was possible. Some of the sibships were too small to be suitable for a chi square analysis, but a test was made on those in which at least five individuals were expected in each of the two classes being compared. In the following sections the results of the tests for heterogeneity are presented if the chi square indicated significance, but not otherwise.

Chi square tests were made for agreement between the observed and expected segregation of the single factors and for independence. Although the joint segregation in each of the experiments is in agreement with the null hypothesis of independence, the possibility exists, of course, that some of the pairs tested are very loosely linked. In order to provide an indication of the rigorousness of the tests for independence, estimates of the recombination fraction, p, and its standard error, s_p, were calculated. Carter and Falconer (1952) have used the recombination fraction less twice its standard error as the "closest admissible linkage." In the present report linkage closer than the .01 fiducial limit of p ($p - 2.326\ s_p$) is considered to be disproved.

The choice of formulas to calculate the chi square for independence and to estimate p and s_p depended upon the segregation of the single factors and upon whether the crosses were made in both repulsion and coupling phases or only in repulsion. When both single factors segregated as expected, the standard formulas were employed (Mather, 1951, Chaps. IV and V). When an aberrant ratio was observed for one of the single factors, calculations were made according to the product formula of Fisher and Balmakund (Mather, 1951: 54-55 and sec. 20) if data in only one phase were available. When one or both single factors gave aberrant ratios and both repulsion and coupling testcrosses were obtained, Fisher's exact treatment of backcross data (Fisher, 1951, sec. 70; Mather, 1951, sec. 21) was applied.

TESTS WITH WALTZER

Waltzer (v), formerly designated "waltzing in bairdi" (Dice, 1935; Watson, 1939), is a recessive gene which produces whirling behavior. The grade of whirling varies among individuals and also varies from time to time within the same individual. Even after the character has been clearly expressed once, some waltzers do not whirl every time they are observed. A few individuals with the genotype vv apparently never display the character.

In order to identify waltzers, mice were checked three times for whirling at one, two, and three months. A display of whirling on any one of the three examinations was considered adequate evidence that a mouse was a waltzer. In each testcross at least 97 per cent of the mice classified as waltzers whirled so rapidly and frequently that no doubt about the identification existed. Some of the testcross progeny which showed no tendency to whirl on any one of the three tests also had the waltzer phenotype. This was shown by rechecking 81 of them. Two (4 per cent) of those retested whirled on the fourth examination.

In each of the crosses with this character fewer than the expected number of waltzers were observed. The proportion of mice which whirled ranged from 35 per cent to 46 per cent, whereas 50 per cent were expected to be waltzers. At least part of the deficiency can be attributed to misclassification of waltzers as nonwaltzers. Whether differential mortality also contributed to it is not known.

The five genes tested with waltzer and the linkage groups to which they belong (McIntosh, 1956), are as follows: flexed-tail (f) in group I, yellow (y) in group II, hairless (hr) in group III, dilute (d) in group IV, and ivory (i), which can not yet be assigned to any linkage group. The tests of flexed-tail and dilute against waltzer were made in part by a triple backcross involving all three of these genes. Double backcrosses supplied the rest of the data for the tests with waltzer. The cross with hairless was made only in the repulsion phase. In this cross good segregation of both single factors was observed, and standard formulas were used to calculate the independence chi square and the estimate of the recombination fraction. The other four crosses with waltzer were made in both repulsion and coupling. In each of them segregation of at least one of the single factors was disturbed, and independence was tested by Fisher's exact treatment of backcross data. The data are summarized in Table I.

Flexed-Tail and Waltzer

Flexed-tail is a recessive gene which produces disalignment of the tail vertebrae and shortens tail length (Huestis and Barto, 1936; Huestis and Lindstedt, 1946). Mice were identified as flexed if, in the absence of evidence of postnatal injury, they exhibited either tail flexures or marked shortening of the tail. Of the 174 mice which were assigned to flexed-tail classes, 170 (98 per cent) exhibited one or both of these symptoms very clearly. The remaining four animals, although lacking obvious flexures, had slight bumps along the tail which could be felt as the tail was drawn between finger and thumb.

Penetrance of flexed-tail is known to be incomplete in some genetic backgrounds and to vary with the degree of expression of the character in the immediate ancestors (Huestis and Lindstedt, 1946). In the testcross for flexed-tail and waltzer a marked deficiency of flexed phenotypes was observed in some sibships from matings in which the double recessive parent expressed the flexed character weakly. In order to reduce the heterogeneity of segregation for flexed-tail, the test for independence was restricted to sibships of the testcross which had either a parent or grandparent with a strongly flexed and very short tail. All sibships which satisfied this requirement were used in the test.

Segregation for flexed-tail in this group is in good agreement with the expected ratio of 1:1, although nonflexed animals slightly outnumber the flexed. Segregation of waltzer, on the other hand, shows a significant deviation from expectation.

The data give no evidence of linkage between flexed-tail and waltzer (χ^2 for independence with one degree of freedom = 0.147; probability = .80—.70). The estimate of the recombination fraction, p, is .5099 with a standard error of .0258. The .01 fiducial limit of p is .450.

TABLE I

Tests of Independence with Waltzer

Gene (x) Tested Against Waltzer (v)	Linkage Group of x	Type of Mating			No. of Sibships	Phenotypes of Offspring					Chi Square			p	s_p	.01 Fiducial Limit of p
		♀		♂		XV	Xv	xV	xv	Total	X : x Ratio	V : v Ratio	Independence			
Flexed . . .	I	xv/xv	x	Xv/xV	5	44	36	33	35	148	2.536	4.435*	0.147	.5099	.0258	.450
		Xv/xV	x	xv/xv	11	20	15	13	11	59						
		xv/xv	x	XV/xv	3	20	5	15	9	49						
		XV/xv	x	xv/xv	4	33	32	32	26	123						
Yellow . . .	II	xv/xv	x	Xv/xV	10	39	29	30	9	107	15.247**	24.894**	0.081	.5086	.0302	.438
		Xv/xV	x	xv/xv	7	52	35	34	16	137						
		xv/xv	x	XV/xv	3	12	10	12	4	38						
		XV/xv	x	xv/xv	5	18	11	19	10	58						
Hairless . .	III	xv/xv	x	Xv/xV	8	19	19	16	19	73	1.636	0.990	0.182	.5152	.0355	.433
		Xv/xV	x	xv/xv	8	40	30	31	24	125						
Dilute . . .	IV	xv/xv	x	Xv/xV	12	40	22	31	25	118	3.609	17.822**	0.014	.5027	.0224	.451
		Xv/xV	x	xv/xv	5	10	10	12	4	36						
		xv/xv	x	XV/xv	21	51	38	51	37	177						
		XV/xv	x	xv/xv	36	148	112	121	96	477						
Ivory . . .	?	xv/xv	x	Xv/xV	6	13	6	13	4	36	1.286	10.321**	0.701	.5411	.0490	.427
		Xv/xV	x	xv/xv	3	12	7	8	8	35						
		xv/xv	x	XV/xv	1	0	0	3	1	4						
		XV/xv	x	xv/xv	7	14	10	10	3	37						

p = estimate of recombination fraction.
s_p = standard error of p.

* indicates significance at the 5 per cent level.
** indicates significance at the 1 per cent level.

Yellow and Waltzer

The yellow mice used in this cross were descendants of the yellow strain " b " of Sumner and Collins (1922). The gene for yellow does not affect the color of the agouti band (McIntosh, 1956), but it reduces the number of all-black hairs and increases the width of the band in the agouti hairs (Sumner and Collins, 1922). If the pigment in the agouti band is yellow, as it was in the strains in which the character was discovered, the mouse looks yellow, with relatively little black ticking (Sumner and Collins, 1922, Pl. 1). The character is variable, and some overlap with the normal condition occurs.

Mice were checked for the yellow character at about one month; most of them were examined again at three months. Identifications usually were made with the naked eye, but if doubt concerning the phenotype existed at the time of the second observation, a small hair sample plucked from the dorsum was checked under the microscope. As the distinction between yellow and nonyellow pelage was greater in the older mice, the second identification was accepted in the few cases in which the first and second differed. Some mice could not be classified with certainty for the yellow character. Sibships containing these animals were not used in the test for independence. Even in the remaining sibships some genetically yellow individuals may have failed to express the character.

Marked deficiencies of yellow mice and of waltzers were observed. The deviation from the expected ratio of 1:1 is highly significant for both characters.

The yellow waltzer class is disproportionately small in both the repulsion and the coupling phase of the testcross. The deviation from proportionality, which is significant in the repulsion phase (χ^2 with one degree of freedom = 4.252), suggests an interaction between yellow and waltzer involving either viability or penetrance. It does not indicate linkage because, although in the repulsion phase the deviation is in the direction which linkage would produce, this is not true of the coupling phase. The test for independence, using both phases of the cross, gives no evidence of linkage (χ^2 with one degree of freedom = 0.081; probability = .80—.70). The estimate of p is .5086 ± .0302, and the .01 fiducial limit of p is .438.

Hairless and Waltzer

Hairless is a recessive gene which greatly reduces the amount of hair in postjuvenile and adult pelages (Sumner, 1924). The expression of the character is variable, but all homozygotes for hairless which survive long enough to shed the juvenile coat can be distinguished readily from normally haired animals. At one month all but one individual of the hairless and waltzer testcross either appeared fully haired or could be identified with certainty as hairless. One mouse which proved to be hairless had a sparse covering of hairs at 32 days, but at two months it had almost no hair other than its vibrissae.

In the testcross for hairless and waltzer, haired mice slightly outnumber hairless ones and nonwaltzers slightly exceed waltzers, but the deviation from the expected ratio of 1:1 is not significant for either character.

Heterogeneity for the recombinant fraction significant at the 5 per cent level was found among sibships in this testcross. (For the eight sibships of 11 or more individuals the heterogeneity χ^2 with seven degrees of freedom is 16.505; probability = .05–.02.) It seems reasonable to assume that sampling errors account for heterogeneity of this magnitude occurring, as it did, in just one of five tests with waltzer.

In the combined data from all sibships the parental and recombinant phenotypes are very nearly equal, as is expected if the genes are independent (χ^2 with one degree of freedom = 0.182; probability = .70–.50). The estimate of p is .5152 ± .0355, and the .01 fiducial limit of p is .433.

Dilute and Waltzer

Dilute is a recessive gene with variable expression. Dilute mice are characterized by a diminution of the black pigment in the hair tips (Dice, 1933) and by uneven distribution of pigment in the proximal region. Here, in some hairs, lumps of pigment and lengths of hair shaft devoid of pigment occur (Clark, 1938). In the dilute mice of the testcross with waltzer, more ventral than dorsal hairs showed the irregularities of pigment distribution.

Identifications of dilute were based on the pigmentation in that part of the hair proximal to the agouti band. The hair bases were exposed by blowing on the fur and were examined with the naked eye. If they did not appear either pale or intensely dark, hair samples were plucked from both the dorsal and ventral pelage and examined microscopically. An identification of dilute was made if the proximal region of the hairs appeared pale to the naked eye or if lumps of pigment were found in hairs of either the dorsal or ventral sample. Of the mice identified as dilute, even those with the weakest expression of the character had several affected hairs in the sample examined. A slight increase in accuracy of identification could have been achieved by microscopically checking hairs from all of the animals in the testcross for dilute and waltzer. In a re-examination of 31 testcross offspring which were classified as nondilute by macroscopic inspection, two (6.5 per cent) were found to have the microscopic characters of dilute animals.

Fewer dilute than nondilute animals and fewer waltzers than nonwaltzers were observed in the testcross. The deviation from the expected ratio of 1:1 is not quite significant for the segregation of dilute, but it is highly significant for waltzer.

The joint segregation of dilute and waltzer is in agreement with the null hypothesis of independence (χ^2 with one degree of freedom = 0.014; probability = .95—.90). The estimate of p is .5027 ± .0224, and the .01 fiducial limit of p is .451.

Ivory and Waltzer

Ivory is a recessive gene which greatly reduces the amount of pigment in both fur and eyes but does not cause complete absence of pigment (Huestis, 1938). The character is sharply distinct from wild-type color in all pelages.

A suggestion of heterogeneity between the sexes for segregation of ivory occurs in the coupling phase of the testcross for ivory and waltzer, in which the one sibship from a heterozygous male consists of four ivory animals, whereas the heterozygous females produced 24 nonivory and only 13 ivory offspring. The probability, calculated by Fisher's exact treatment of 2 x 2 tables (Fisher, 1938, sec. 21.02), of getting such disparate segregations by chance alone is only .023. But as neither distribution differs significantly from the expected 1:1 ratio, the excess of ivory in one case and the deficiency in the other are assumed to be fortuitous. In the combined data from coupling and repulsion phases, segregation of ivory is close to that expected.

Waltzer, as in the other crosses involving this character, appears in fewer than the expected numbers. The deviation is highly significant.

Ivory and waltzer appear to segregate independently (χ^2 for independence with one degree of freedom = 0.701; probability = .50—.30). The estimate of p is .5411 ± 0.0490, and its .01 fiducial limit is .427.

Discussion

No evidence was found for linkage between waltzer and any of the five genes: flexed-tail, yellow, hairless, dilute, and ivory. If any linkage does exist between any of them and waltzer it must be quite loose with more than 40 per cent crossing over. In the following discussion two genes which have been tested directly against each other are stated to be independent if, as in the five tests with waltzer given above, the .01 fiducial limit of the recombination fraction is greater than .40.

A test of independence of one linkage group from another usually involves testing both terminal genes of one group against both terminal genes of the other, but the linkage between waltzer and wide-band is so close that the small increase in precision gained by tests with both of these genes rather than with one hardly justifies the additional expenditure of time involved. After the discovery of close linkage between wide-band and waltzer, other genes were tested with only one of the two. Before this discovery tests of some genes were completed with both wide-band and waltzer.

In linkage group I of Peromyscus, which includes albino, pink-eye, silver, and flexed-tail, the order of the genes has not been ascertained. The loosest linkage yet reported in this group is that between albino and flexed-tail with crossing over of about 41 per cent in females and about 30 per cent in males (Huestis and Lindstedt, 1946), and these two genes may be the terminal ones among those so far identified. In a previous test waltzer was shown to segregate independently from albino (Barto, 1942), and in the present report its independence from flexed-tail is demonstrated. This seems adequate evidence for basing a tentative claim of independence of the group consisting of waltzer and wide-band from group I. Additional tests against group I will be desirable if either pink-eye, which is linked to albino with crossing over of about 20 per cent in females and about 13 per cent in males (Clark, 1938), or silver, which is linked to flexed-tail with crossing over of about 27 per cent in females and 18 per cent in males (Huestis and Lindstedt, 1946), proves to be terminally located.

Only a single gene, yellow, is known for linkage group II, and group III also is marked by only one gene, hairless. Both yellow and hairless were previously shown to segregate independently from wide-band (Clark, 1938; McIntosh, 1956), and they are here demonstrated to have random assortment with waltzer. Group IV has two markers, dilute and brown-tip, with crossing over between them of about 43 per cent in females and about 40 per cent in males (McIntosh, 1956). Random assortment was demonstrated by McIntosh (1956) between waltzer and brown-tip and is here shown between waltzer and dilute. As independence of groups I and IV from waltzer and of groups II and III from both waltzer and wide-band has been demonstrated, these two linked genes are established as linkage group V. It should be noted, however, that any statement of independence of a group is necessarily tentative because each discovery of new linkages extending the known length of any group makes more rigorous tests possible.

Ivory, by the demonstration of its independence from waltzer, is excluded from group V. It was previously shown to segregate independently from yellow of group II (McIntosh, 1956), from hairless of group III, and from dilute of group IV (Barto, 1942). Tests against group I and against brown-tip of group IV remain to be made before ivory becomes a marker.

TESTS WITH EP

The character formerly called " waltzing and epilepsy in artemisiae " (Dice, 1935; Watson, 1939) is here specified by the letters EP in order to avoid confusion between it and the trait now designated " waltzer " (see section on Tests with Waltzer). The EP syndrome consists of whirling, sound-induced convulsions, and progressive loss of hearing (Dice, 1935; Watson, 1939; Chance and Yaxley, 1949; Ross, 1953). The gene, e, which determines it is recessive. Not all of the affected animals exhibit the complete syndrome, and a small proportion of mice having the genotype ee fails to display either whirling or convulsions. Expression also varies from time to time within the same individual.

Mice were checked for the EP syndrome by exposing them to the sound of jingling keys. The duration of the key jingling on each test was at least 90 seconds. Two or three tests were given, at least one week apart, with a total of at least 270 seconds of key jingling. Mice were identified as EP if they exhibited either whirling or convulsions on any one of the tests. The testing procedure was not adequate to identify quite all of the EP animals. In a recheck of 26 mice from a backcross for EP which were assigned to the non-EP class on the basis of their behavior during three tests, one individual had a severe seizure and so proved that it had the EP syndrome. In the crosses with EP the number of mice which whirled or had seizures was from 73 to 99 per cent of the expected number of EP animals. Misclassification of EP as non-EP undoubtedly accounts for at least part of the observed deficiency, and perhaps for all of it. Whether differences in viability exist between the two phenotypes is not known.

EP was tested against albino (c) and silver (si), in linkage group I, and against brown-tip (b) in group IV. The data for these three tests are summarized in Table II.

There is a tendency for the symptoms of the EP offspring to resemble those of the closest EP ancestor. Thus, in the testcross generation of EP and albino, which was produced largely by matings in which the EP parent exhibited both convulsions and whirling, more than two-thirds of the EP animals displayed both these symptoms. Most of the testcross with brown-tip came from matings with convulsives which failed to whirl, and more than two-thirds of the EP mice of this testcross were convulsive nonwhirlers. Silver was crossed with convulsives which did not whirl and with whirlers which had no convulsions. In the F_2 generation all the EP animals from the cross with convulsives displayed seizures; half of the EP mice from the cross with whirlers exhibited whirling without seizures. But within each of the crosses to albino, silver, and brown-tip, the proportion of offspring which displayed at least one of the EP symptoms is essentially the same whether the matings involved convulsive whirlers, convulsive nonwhirlers, or nonconvulsive whirlers. These findings are in

TABLE II

Tests of Independence with EP

Gene (x) Tested Against EP (e)	Link-age Group of x	Type of Mating			No. of Sib-ships	Phenotypes of Offspring					Chi Square			p	s_p	.01 Fiducial Limit of p
		♀		♂		XE	Xe	xE	xe	Total	X : x Ratio	E : e Ratio	Inde-pendence			
Albino . .	I	xe/xe	x	Xe/xE	5	11	4	12	8	35	0.766	5.281*	0.012	.5031	.0272	.440
		Xe/xE	x	xe/xe	21	79	65	86	69	299						
Silver . .	I	Xe/xE	x	Xe/xE	13	137	32	43	8	220	0.388	5.455*	0.278	.4679	.0523	.346
Brown-tip Group A	IV	xe/xe	x	Xe/xE	8	28	24	53	37	142	10.169**	2.817	0.342	.4744	.0438	.372
Group B		Xe/xE	x	xe/xe	6	28	25	31	26	110	0.246	0.005	0.608	.5276	.0354	.445
		xe/xe	x	XE/xe	2	8	5	6	6	25						
		XE/xe	x	xe/xe	3	12	25	15	12	64						

p = estimate of recombination fraction.
s_p = standard error of p.
* indicates significance at the 5 per cent level.
** indicates significance at the 1 per cent level.

agreement with the conclusion of Watson (1939) that one major gene determines the EP abnormality but that its expression is subject to modification by other genes.

Albino and EP

Albino is a recessive gene which results in absence of pigment from both fur and eyes in homozygotes (Sumner and Collins, 1922). Its penetrance is complete.

The test of EP with albino was made on a repulsion testcross. Good segregation was observed for albino, but for EP the deviation from expectation is significant at the 5 per cent level.

The joint segregation of EP and albino is in agreement with the null hypothesis of independence (χ^2, calculated by the product formula = 0.012, with one degree of freedom; probability = .95—.90). The estimate of p is .5031 ± .0272, and the .01 fiducial limit of p is .440.

Silver and EP

Silver is a recessive gene (Huestis and Barto, 1934) which produces an effect very similar to that of the gene dilute (see section on Dilute and Waltzer). Mice were checked for this character by examining the bases of dorsal hairs. Most of the identifications were made with the naked eye, but in doubtful cases a hair sample was examined with the microscope.

The test between silver and EP was made on an F_2 generation. The data for this test, collected incidental to another study, are less extensive than those for the other tests reported here, but are adequate to disprove close linkage and therefore seem worth recording.

The observed proportion of nonsilver and silver mice is very close to the expected 3:1 segregation. Fewer than the expected one-fourth of EP mice were observed. The deviation is significant at the 5 per cent level.

Because of the disturbed segregation of EP, the product formula was used to test independence. Silver and EP appear to be segregating independently (χ^2 for independence, with one degree of freedom = 0.278; probability = .70—.50). The estimate of the recombination fraction is .4679 ± .0523, and the .01 fiducial limit of p is .346.

Brown-tip and EP

The brown-tip animals used in the linkage test were descended from a stock of P. m. blandus from Tularosa, New Mexico. The brown-tip gene in the Tularosa stock was shown (Blair, 1947) to be at the same locus as a gene previously discovered in the subspecies P. m. gambeli from Silver Lake, Oregon, and designated " brown " (Huestis and Barto, 1934). Brown-tip is a recessive gene which affects the sepia pigment granules of the fur in all pelages. In brown-tip mice these granules are usually brown, whereas in mice with the alternative black-tip allele they appear either black or very dark brown. The brown-tipped animals in the linkage test all appeared sharply distinct from the black-tipped ones.

Heterogeneity for segregation of brown-tip was found among mating types in the cross of brown-tip and EP (χ^2 for heterogeneity, with three degrees of freedom = 9.175; probability = .05–.02). The observed distribution of black-tip and brown-tip animals is close to the expected 1:1 ratio in the coupling phase of the testcross and in the repulsion testcross obtained from segregating females (group B), but a deficiency of black-tipped animals occurs in the repulsion testcross from heterozygous males (group A). The deviation from expectation in group A is highly significant. In previous studies of the brown-tip locus (Huestis and Barto, 1934, 1936; Blair, 1947) the observed segregation, with one exception, is in good agreement with expectation. In one backcross (Huestis and Barto, 1934) the class with wild-type color is deficient, as it is in group A here. Although the nature of the disturbance is not certain, differential viability seems more likely to be involved than failure of penetrance, as penetrance of brown-tip has always proved to be complete when tested by mating brown-tip mice together.

Slightly fewer EP than non-EP mice were observed in the testcross with brown-tip, but the deviation from a 1:1 ratio is not significant, either in group A, or in group B, or for the two groups combined.

Because of heterogeneity in segregation of brown-tip, groups A and B were tested separately for independence. The product formula was used for group A, in which poor segregation of brown-tip was observed. The standard formulas were used for group B, in which both single factors segregated in good agreement with expectation.

Neither group gives any evidence of linkage between brown-tip and EP. The estimates of the recombination fraction in groups A and B are reasonably close. The estimate from group B, .5276 ± .0354, is the more precise of the two, and the fact that it is based on combined coupling and repulsion data is another point in its favor. The .01 fiducial limit of this estimate is .445. A calculation of the recombination fraction and its standard error from the combined data of groups A and B gives a slightly higher fiducial limit.

Discussion

The tests of EP with albino, silver, and brown-tip are all in agreement with the null hypothesis of independence. Linkage of 40 per cent and closer is disproved between EP and albino and between EP and brown-tip. Linkage of .34 per cent and closer is disproved between EP and silver. EP has not been tested with any genes but the three reported here and can not yet be excluded from any linkage group.

SUMMARY

The following pairs of genes in Peromyscus maniculatus either are independent or, if any of them are linked, have a recombination fraction higher than 40 per cent: waltzer (v) and flexed-tail (f), waltzer and yellow (y), waltzer and hairless (hr), waltzer and dilute (d), waltzer and ivory (i), EP (e) and albino (c), and EP and brown-tip (b).

Ep and silver (si) either are independent or, if linked, have a recombination fraction higher than 34 per cent.

The linkage group consisting of wide-band (Nb) and waltzer is independent from the other four known linkage groups in Peromyscus maniculatus and so constitutes group V for this species.

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